Rapid Jumps and Bioluminescence Elicited by Controlled Hydrodynamic Stimuli in a Mesopelagic Copepod, *Pleuromamma xiphias*

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Abstract. Actively vertically migrating mesopelagic copepods are preyed upon by a wide variety of fishes and invertebrates. Their responses to predatory attacks include vigorous escape jumps and discharge of bioluminescent material. Escape jumps and bioluminescent discharges in the calanoid copepod *Pleuromamma xiphias* were elicited by quantified hydrodynamic disturbances. Brief weak stimuli (peak water velocity $64 \pm 21 \ \mu\text{m s}^{-1}$) elicited weak (peak force 6.5 dynes) propulsive responses ("jumps") and no bioluminescence. Moderate stimuli ($1580 \pm 780 \ \mu\text{m s}^{-1}$) produced strong propulsive responses consisting of long trains of coordinated power strokes by the four pairs of swimming legs ("kicks"). Peak forces averaged 42 dynes. Strong stimuli ($5520 \pm 3420 \ \mu\text{m s}^{-1}$) were required to elicit both a jump and a bioluminescent discharge. In several cases, multiple stimuli were needed to evoke bioluminescence, given the limits on stimulus magnitude imposed by the apparatus. Repeated bioluminescent discharges could be evoked, but this responsiveness waned rapidly. Latencies for the jump response (14 ± 4 ms) were shorter than for the accompanying bioluminescent discharge (49 ± 26 ms). The higher threshold for eliciting bioluminescent discharge compared to escape jumps suggests that the copepods save this defense mechanism for what is perceived to be a stronger threat.

Introduction

Planktonic copepods are preyed upon by a wide variety of fishes and invertebrates (Hopkins and Baird, 1985; Hopkins *et al.*, 1996). Thus, predator evasion strategies are key to the survival of these animals in pelagic communities. Planktonic copepods respond to perceived attacks with rapid and powerful escape "jumps" (Singarajah, 1969, 1975; Strickler, 1975). The Augaptiloidea (Calanoida), which typically inhabit the mesopelagic region, possess the ability to discharge bioluminescent material (Clarke *et al.*, 1962; Herring, 1988). These discharges are thought to either startle a potential predator away or misdirect a possible attack (David and Conover, 1961; Morin, 1983; Young, 1983). Although we have a qualitative understanding that bioluminescent discharges in these calanoids are used as a defense mechanism, we know less about how these discharges are triggered in the natural environment. In the laboratory, electrical stimulation and mechanical agitation are routinely used to elicit bioluminescent discharges (*e.g.*, Latz *et al.*, 1987, 1990; Widder, 1992). However, we know little about the magnitude of stimuli required to elicit this behavior. Neither do we understand the relationship between the escape jump and the bioluminescence. We addressed some of these questions in a laboratory study, working with tethered *Pleuromamma xiphias*. This calanoid is a metridinid (Augaptiloidea) and belongs to a widespread and abundant genus in this group. Here we report on the minimum hydrodynamic stimuli necessary to elicit a jump response, and how this compares to the minimum stimulus that triggers bioluminescence. By concurrently monitoring jump behavior with a force transducer and bioluminescence with

a photomultiplier tube, we are able to describe the temporal sequences for the two behaviors following a quantitative stimulus.

Materials and Methods

Collection

Animals were collected at night (2000 to 2200 h), about 1 mile offshore from Keauhou Bay, Kona, Island of Hawaii, at a depth of 70 to 100 m. A plankton net (0.5-m diam, 333-μm mesh) was towed from a small boat at idle speeds (<2 knots) for 15 to 20 min. Within 2 h of collection the animals were sorted into jars with clean seawater, cooled to 6°C, and flown, in coolers, to Oahu. Once the animals were brought into the laboratory (within 16 h of collection), they were kept in the dark at 6° to 8°C. Every 2 to 3 days the copepods were fed under dim red light with a mixture of *Artemia* nauplii and *Isochrysis galbana* cells.

Tethering

Copepods were affixed to aluminum wire tethers with cyanoacrylate glue (Borden or Loctite) under red light in an otherwise darkened room. They were corralled in a droplet of seawater, which was then drawn down until a portion of the dorsal prosome was briefly exposed to air. The wire, with some glue on its tip, was applied and held in place while the animal was reimmersed. During this procedure the animals typically bioluminesced in response to the tactile stimulation. Once a copepod was glued and transferred to the experimental setup, 3 h were allowed to elapse before it was tested for mechanical sensitivity. Good experimental animals had high mechanical sensitivity, maintained their swimming appendages in the promoted position (tucked under the body, anteriorly directed), and were bioluminescently competent. In the experiments presented, the animals maintained their mechanical sensitivity for at least 2 days, although the force produced during the jump typically declined. Toward the end of the experiments we observed either a loss in sensitivity or a failure to maintain the swimming appendages in the promoted position. All animals were still bioluminescently competent at the end of the experiments and responded to direct tactile stimulation with a discharge. While on the tether, copepods were fed *Isochrysis galbana*.

Health

To test the bioluminescent competence of *P. xiphias*, five specimens were tested for total mechanically stimulable luminescence (TMSL) using methods described in Buskey and Swift (1990). A single *P. xiphias* was placed in each of five liquid scintillation vials containing 10 ml of filtered seawater. After allowing the copepods to recover for about 2 h from the disturbance of being transferred, we placed a vial inside an integrating sphere (Labsphere, Polane coated) and stimulated bioluminescence by stirring the vial with a battery-powered test-tube stirrer until no additional bioluminescence was detected. Bioluminescence was quantified using a photomultiplier tube (PMT; Hamamatsu R464) and a photon-counting photometer (Hamamatsu C1230). Values for TMSL of *P. xiphias* ranged from 5.3×10^{10} to 5.5×10^{11} photons, with a mean of 2.4×10^{11} photons. These results are similar to previously measured values of TMSL for *P. xiphias* (Buskey and Swift, 1990; Latz *et al.*, 1990) and indicate that our experimental animals were capable of full bioluminescence and were in good physiological condition.

Experimental protocol

The experimental setup is diagrammed in Figure 1 and is described in detail in Lenz and Hartline (1999). After the tethered copepod was positioned in the apparatus, red background lights were turned off, and illumination was switched to infrared light from four Optek OP-293A LEDs emitting 875 ± 20 nm and positioned about 1 cm behind the animal, outside of the field of view of the video camera. Hydrodynamic stimuli were generated using a piezoelectric pusher to control movement of a plastic sphere of either 3- or 5-mm diameter positioned about 3 mm in front of the animal. At maximum amplitude, the experimental sphere was displaced vertically by 40 μm. A behavioral response was elicited at threshold by vertical movements of the larger sphere of less than 0.5 μm. Water displacement at the rostrum, approximately parallel to the long axes of the first antennae, was calculated based on the dipole attenuation expected of near-field laminar water flow (Kalmijn, 1988; Gassie *et al.*, 1993). Although there are some errors and approximations inherent in this indirect approach to determining stimulus magnitude (see Gassie *et al.*, 1993, and Lenz and Hartline, 1999, for detailed discussion), it is widely used in behavioral and physiological studies on hydrodynamic reception in aquatic organisms (*e.g.*, Coombs *et al.*, 1989; Bleckmann, 1994; Coombs, 1994) and provides a reasonable measure given uncertainties in such factors as the location of receptors. Computer-controlled stimuli included short and long sinusoidal movements ranging in frequency from 50 to more than 1000 Hz.

Force measurement

During a rapid swim the copepod exerted a force on the tether. The displacement this produced along a horizontal axis, roughly parallel to the copepod's body axis, was measured with a fiberoptic displacement sensor (Philtec 88N) positioned opposite to a small reflective disk mounted on the tether (Fig. 1). The force was calibrated by pushing
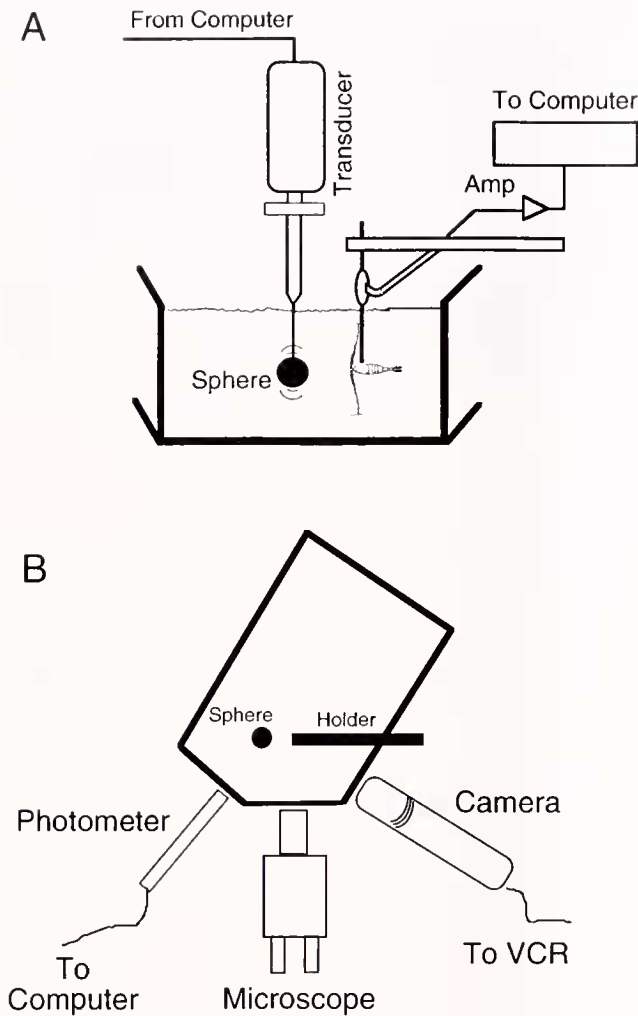
A

B

Figure 1. Diagram of the experimental setup. (A) Side view showing the positions of the dipole (sphere) and the glued copepod. The sphere used in the stimulus was either 3 or 5 mm in diameter. The distance between the center of the sphere and the rostrum of the animal ranged from 3 to 5 mm. (B) View from the top. Location of the photometer, dissecting microscope, and camera are shown. The experimental dish was made out of microscope slides and designed to allow positioning of the equipment at right angles to the glass.

against the tether with a wire, the deflection of which had been calibrated using weights. Force-transducer responses were monitored with an oscilloscope, digitized at 42 kHz per channel, and stored on computer. Resonance frequency of the transducer (1.5-2 kHz) was kept as high as possible while maintaining sufficient sensitivity for measurements. The transducer was underdamped, with an overshoot of around 20% to abruptly applied (0.5 ms rise) forces; it had a damping time-constant of 4 ms. Force signals were filtered at 2 kHz with an 8-pole Bessel filter. Further details of the recording system are given in Gassie *et al.* (1993) and Lenz and Hartline (1999).

Optical train

Mounted perpendicular to the view presented in Figure 1A were a photometer, a dissecting microscope, and a video camera (Fig. 1B). Each of these instruments faced one of the five sides of the experimental chamber. Light from the I-R LEDs was blocked from the photometer with an interference filter (center wavelength 480 nm), and background recordings were very low. The spatial and temporal patterns of bioluminescent emission of *P. xiphias* were recorded on videotape using a Cohu monochrome CCD (charge-coupled-device) camera (30 fps) fitted with a 55-mm Micro-NIKKOR macro lens, coupled to a Videoscope International KS-1381 microchannel plate image intensifier. The video output signal was recorded on a Mitsubishi HU-770 videocassette recorder. The stimulus-trigger from the computer also triggered a 30-ms-long flash in an I-R LED, producing a single video frame with an elevated light level. This was used to correlate video with force and PMT records, which thus had an uncertainty of 30 ms. Characteristics of the bioluminescence monitored by the PMT could frequently be used to estimate the relative timing with higher temporal resolution.

Light measurement

The bioluminescent emissions of *P. xiphias* were measured in two ways: with a photomultiplier photometer and with an image intensifier. In early experiments, photometer measurements were made using a Hamamatsu C1230 photon counter and a Hamamatsu R464 PMT. This system was convenient for measuring the total integrated bioluminescence emitted by *P. xiphias*, but it did not provide the temporal resolution necessary to accurately measure flash kinetics since it integrates counts over 0.1-s intervals. It was replaced with a Pacific Instruments model 126 wide-range photometer using an EMI QL-30 PMT. Amplified voltage from the PMT was sent directly to the computer and digitized along with other components of the data stream. Before and after being shipped to Hawaii, both photometer systems were calibrated using cultures of bioluminescent bacteria (*Photobacterium* sp.) and a calibrated Quantalum 2000 luminescence photometer with a highly stable silicon photodiode sensor. A secondary standard (^{14}C emission standard made from Sylvania Type 132 blue phosphor, peak wavelength 455 nm) was also calibrated. The secondary standard was measured frequently to allow for calibration of readings of bioluminescence.

Results

Sudden hydrodynamic disturbances were capable of eliciting behavioral responses in *Pleuromamma xiphias*; we interpret these responses as "rapid swims," or "jumps." In

tethered animals, a complex temporal pattern of force development followed closely on the presentation of such a stimulus. Figure 2A shows a typical response to a brief (2-ms) water movement of peak-to-peak amplitude computed at 3.83 μm at the copepod's rostrum. Following a short latency ("L"), there was an abrupt rise ("R") in forward propulsive force. Then a relatively rapid return past zero force to a smaller reverse force ("Rv") led to the development of a second forward component. As in a previous study on the epipelagic copepod *Undinula vulgaris* (Lenz and Hartline, 1999), we interpret these propulsive units to be kicks generated by the combined power strokes of the four pairs of swimming legs (pereiopods). The features of strong locomotor responses in *P. xiphias* were
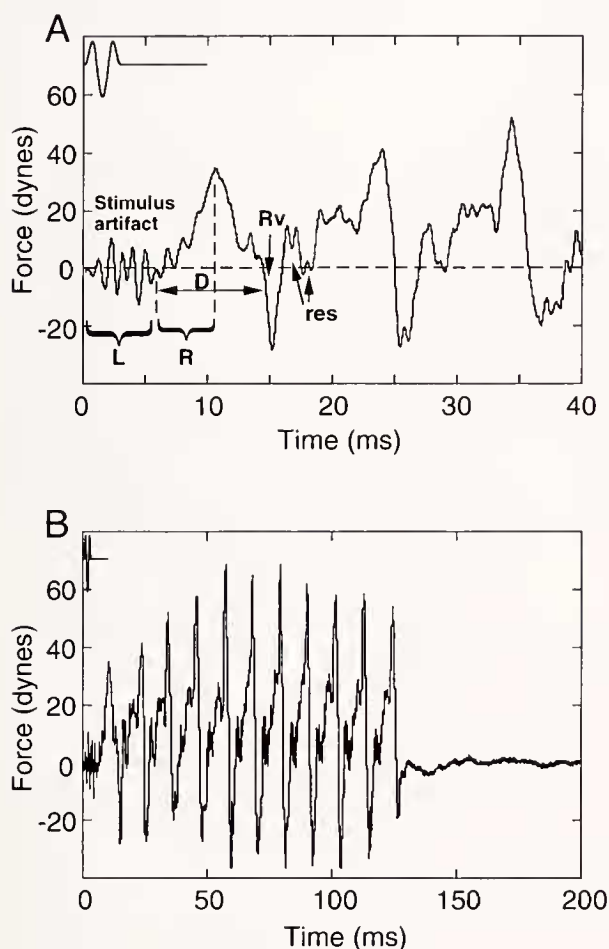

Figure 2. Force record of a fast swim response of a *Pleuromamma xiphias* adult female to a suprathreshold hydrodynamic stimulus. (A) Expanded temporal scale showing the first four kicks of the response. (B) Record showing the complete response to the stimulus. Stimulus: 700 Hz, 1.5 cycles, maximum water velocity of 8400 μm s^{-1} at the rostrum. Piezoelectric transducer: PZL-060; vertical movement of sphere: 40 μm; sphere diameter: 5.1 mm; distance from center of ball to rostrum: 4.4 mm (BPL97-8.D04, second trace).

similar in most respects to those of *U. vulgaris* (Lenz and Hartline, 1999). They were characterized by short latencies, measured from the onset of the stimulus to the onset of the forward propulsion, typically around 10 ms (minimum: 6 ms). A weak brief backward propulsion, or "preparatory movement," was observed in some animals immediately preceding the forward propulsion (*e.g.*, Fig. 3A, "Pr"). Following the peak of forward propulsion, there was often an irregular pattern of peaks and valleys for the remainder of the short stroke duration (mean: 8.7 ms, Table I). As in *U. vulgaris* (Lenz and Hartline, 1999) and *Calanus helgolandicus* (Svetlichnyy, 1987), the major peaks can be assigned to the individual strokes of pereiopod pairs. Minor peaks caused by resonance in the underdamped force-transducer system were also often apparent (Fig. 2A "res"). The distinct reverse propulsion following the termination of the forward phase was a feature found consistently in *P. xiphias* but not in previous studies on *U. vulgaris*. A pattern of multiple kicks in quick succession characterized a strong response to a stimulus. This is illustrated in Figure 2B, which shows the same response as Figure 2A on a compressed time scale. In *P. xiphias*, a train of kicks was typical, producing a cohesive propulsive response we term a "jump." Within the train, kicks occurred at repetition rates of 80 Hz (Table I; range 59 to 98 Hz).

Response depended on stimulus magnitude

With the experimental setup described, we were able to monitor jumps and bioluminescence simultaneously. As with other copepods we have tested, *P. xiphias* is very sensitive to water movement. Figure 3 shows records from the PMT and the force transducer at three stimulus intensities. We observed several degrees of response, graded with the intensity of the stimulus (Table II). Figure 3A shows a "weak" response given to the lowest intensity of a 1.5-cycle stimulus that elicited a measurable response in this animal. Peak water velocity produced by this stimulus at the rostrum was calculated to be 50 μm s^{-1} (BPL97-10; Table II). Neither the PMT nor the image intensifier recorded any sign of bioluminescence. The force trace shows first one small 12-dyne kick followed by a 100-ms delay and then three additional kicks. The cumulative force impulse generated by these kicks (the integral of force over time; related to total distance moved in a linear viscous medium) reaches only 0.2 dyne-second. In general, a weak response consisted of a brief force transient, which often barely registered on the force transducer (*e.g.*, mean of 6.5 dynes, Table I). These weak responses consisted of a small number of propulsive events (*e.g.*, 1–3) with moderate latencies (15–20 ms). We term them "weak kicks," but determining what is involved in their production awaits high-resolution cinematography. As in Figure 3A, a weak kick was sometimes
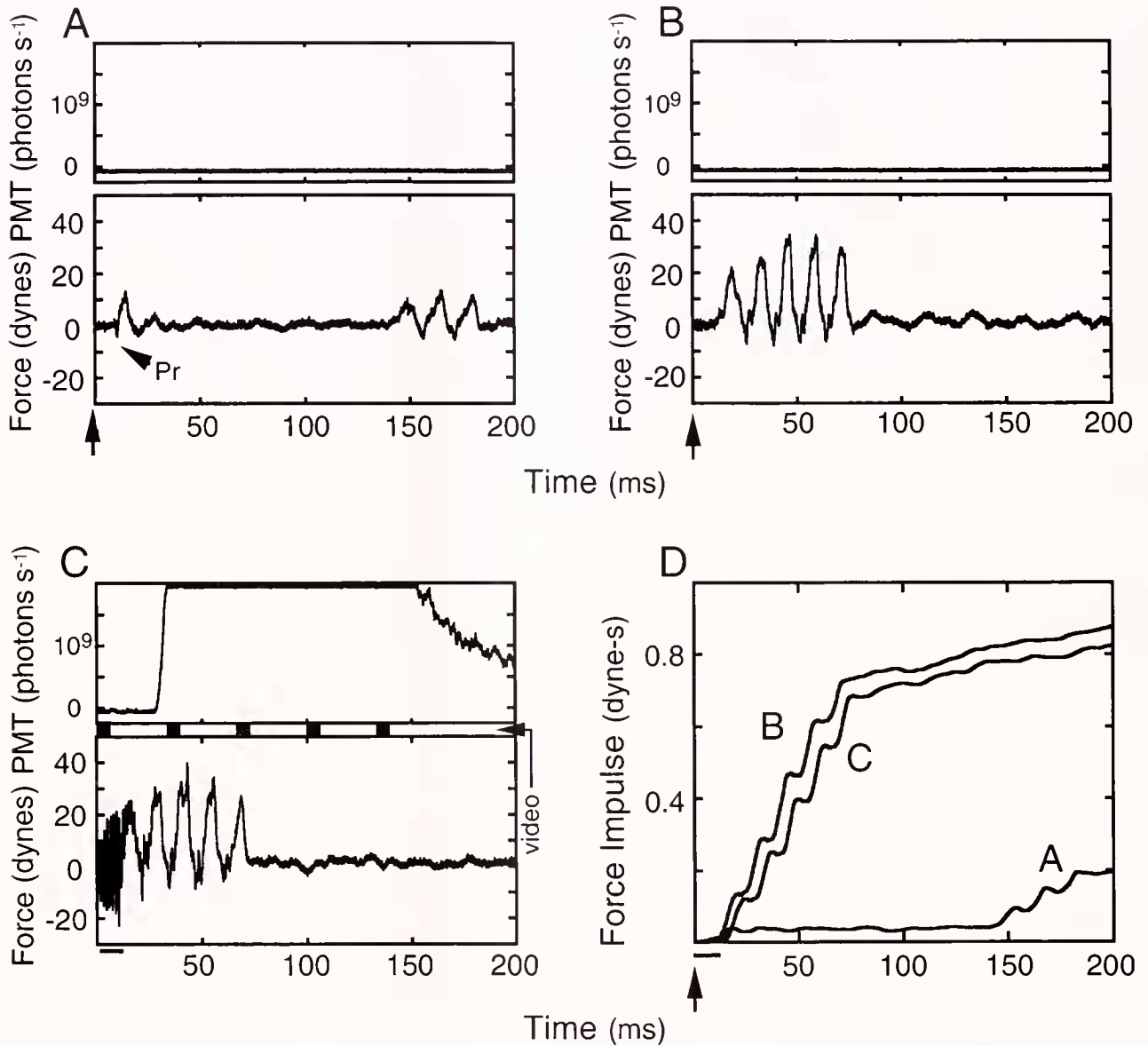
Figure 3. Behavioral responses of a *Pleuromamma xiphias* adult female to three stimulus intensities. Hydrodynamic stimulus was produced by a piezoelectric transducer (PZL-060) with a 5.1-mm sphere, the center of which was located 4.3 mm from the animal's rostrum. (A) Photomultiplier tube (PMT) and force records showing response to a small stimulus: 700 Hz, 1.5 cycles, maximum water velocity of 50 μm s^{-1} at the rostrum (vertical peak-to-peak movement of sphere: 0.22 μm). (B) PMT and force records showing response to a moderate stimulus: 700 Hz, 1.5 cycles, maximum water velocity of 1600 μm s^{-1} at the rostrum (vertical peak-to-peak movement of sphere: 7.1 μm). (C) PMT and force records showing response to a large stimulus: 700 Hz, 8 cycles, maximum water velocity of 8900 μm s^{-1} at rostrum (vertical peak-to-peak movement of sphere: 40 μm). Response is to fourth stimulus in a series of five presented at 1-s intervals (the animal also luminesced to the fifth presentation). Estimated times of video frames shown in Figure 5 indicated with marks along the time axis. (D) Integral of force over time for the force records shown in A, B, and C. Arrows indicate stimulus presentation. Bar in C indicates the length of time the stimulus was on (11.5 ms). Stimulus length in A and B: 2 ms (BPL97-10.D02, D04, D06).

followed by a 50–200 ms period of quiescence and then a cluster of delayed, sometimes stronger, kicks.

As stimulus intensity was progressively increased above the threshold level, a point was passed at which the intensity of the response increased abruptly (Tables I, II). Figure 3B shows force and PMT records for a stimulus intensity that is

Table I

Characteristics of escape response elicited by a hydrodynamic stimulus

Experiment	Sex	Weak kick force (dynes)	Max kick force (dynes)	Latency (ms)	Kick duration (ms)	Kick frequency (Hz)
BPL97-3	M	7.5 ± 1.7 (5)	60.0 ± 6.4 (5)	12.9 ± 9.1 (6)	8.1 ± 1.2 (4)	89 ± 3 (5)
BPL97-6	M	4.7 (1)	24.8 ± 6.8 (5)	11.5 ± 1.0 (6)	6.9 ± 0.6 (5)	98 ± 5 (6)
BPL97-8	F	4.3 (1)	55.9 ± 3.5 (6)	7.4 ± 0.6 (6)	8.5 ± 0.7 (6)	89 ± 5 (6)
BPL97-10	F	10.4 ± 3.2 (8)	36.8 ± 6.8 (19)	11.1 ± 3.3 (16)	11.3 ± 0.8 (8)	75 ± 4 (8)
BPL97-11	F	5.5 ± 1.5 (3)	34.2 ± 6.8 (4)	15.6 ± 1.7 (4)	8.8 ± 2.4 (6)	56 ± 5 (7)

Weak kick forces were measured from escape responses to near-threshold stimuli. Maximum kick force, latency, kick duration, and kick frequency were all measured from responses to suprathreshold stimuli. Maximum kick force refers to the largest force produced in a train of kicks. Latency and kick duration were measured as shown in Figure 2 (L, D). Kick frequency was calculated by averaging the number of kicks over time either for the complete jump or over the data record (200 ms) in the cases where the jumps extended beyond the sampling window. Means and standard deviations are given; sample size (in parentheses) indicates the number of measurements used for the mean and SD.

30 times higher than that shown in Figure 3A. The PMT record shows no sign of bioluminescence. However, many characteristics of the force record are substantially augmented (Fig. 3B). The response typically involved multiple strong kicks with maximum forces produced by individual kicks registering nearly 40 dynes (Fig. 3B). The force impulse produced in the example shown in Figure 3B over a 75-ms interval approached 1 dyne-second (Fig. 3D). In general, such "strong" responses were elicited at stimulus strengths 15 to 30 times above threshold for the weak kicks (Table II). Peak amplitudes (mean = 42 dynes) were greater by a factor of 5 or more than for the weak kicks (Table I).

Duration of individual kicks averaged 8.7 ms (Table 1). The overall envelope of peak forces during a jump was "spindle" shaped (Fig. 2B). The first few kicks increased progressively in amplitude, then continued with several (sometimes 35 or more) kicks, and finally tapered off somewhat before ending. Figure 4 shows an example of the initial phase of one of these very long spindle-shaped jumps. In a multiple-stimulus protocol, the first or second stimulus of a train of five at 1.5-s intervals usually evoked the longest spindle-shaped jump.

Further increase in stimulus intensity would in some cases result in a bioluminescent discharge. Figure 3C shows

Table II

Calculated water velocities that elicited behavioral responses: weak kicks, strong kicks, and strong kicks and bioluminescent discharges

Expt	Sex	Weak kick response		Strong kick response		Jump + biolum response	
		Stim	Velocity (μm s^{-1})	Stim	Velocity (μm s^{-1})	Stim	Velocity (μm s^{-1})
BPL97-3	M	S700	70	S700	2220	S700	2220
BPL97-6	M	S700	66	S700	1170	S700	6590
BPL97-7	F	ND	ND	ND	ND	S700	8420
BPL97-8	F	S700	84	S700	840	ND	ND
BPL97-9	F	S700	28	S700	890	ND	ND
BPL97-10	F	S700	50	S700	1570	F700	8860
BPL97-11	F	S700	89	S700	2770	F700	8860
BPL97-1	F	F700	58	ND	ND	F700	1830
BPL96-1	M	ND	ND	ND	ND	F700	1830
Mean			64		1580		5520
SD			21		780		3420

Water velocities at the copepod were calculated using dipole equations. Sinusoidal vertical movements of sphere at 700 Hz were either short (S700, 1.5 cycles) or long (F700, 8 cycles). ND = not determined; threshold could not be established.
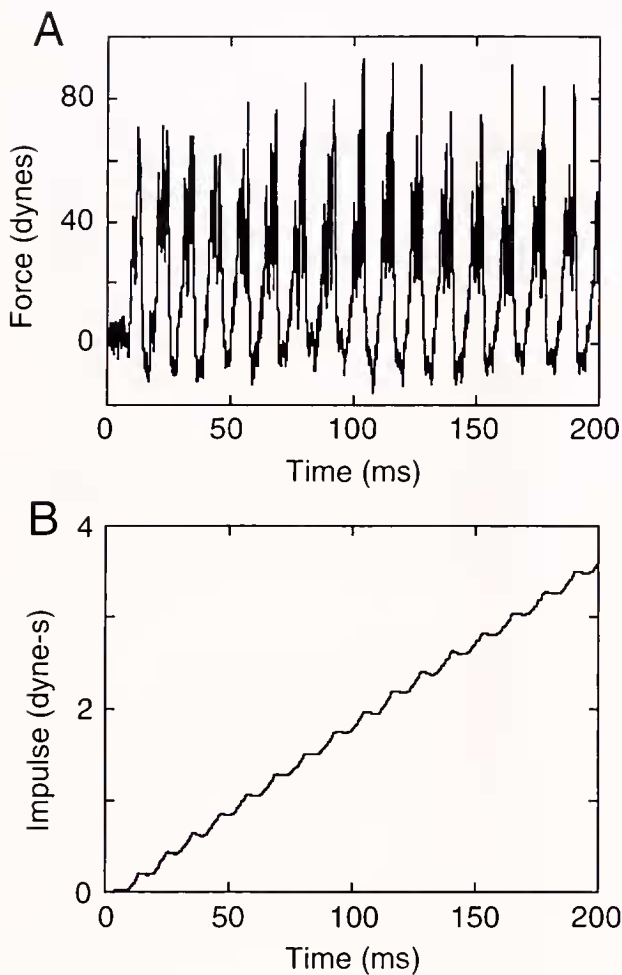
A

B

Figure 4. (A) Force record of a long series of multiple kicks in response to a large stimulus in *Pleuromamma xiphias*, adult female. (B) Integral of force over time for the force record shown in A. Stimulus: 700 Hz, 1.5 cycles, maximum water velocity of 8900 μm s^{-1} at rostrum. Piezoelectric transducer: PZL-060; sphere diameter: 5.1 mm; distance from center of ball to rostrum: 4.4 mm; vertical peak-to-peak movement of sphere: 40 μm.

the response of the same animal as in Figures 3A and 3B to a stimulus with an amplitude 180 times greater (and of longer duration) than threshold for eliciting the weak jump. Both a jump and a bioluminescent discharge were produced. The jump response was initiated well before (18 ms) the bioluminescence (Fig. 3C, top panel). The bioluminescent discharge started at the end of the second kick in a train of five and lasted for about 200 ms. The integrated force for the train of kicks was about 0.8 dyne-second (Fig. 3D). Bioluminescence was usually accompanied by strong spindle-shaped jumps. Near threshold for bioluminescence, discharges were likely to be given in response to one of the later stimuli in a sequence of five, and were thus not clearly associated with the strongest (=longest) jump.

We were not able to elicit bioluminescent discharges to hydrodynamic stimuli in all cases. This was not due to a lack of bioluminescent competence, as electrical stimuli or more vigorous mechanical disturbance would invariably elicit bioluminescence even if our strongest hydrodynamic stimulus would not. In five experiments, we obtained thresholds for both jump and bioluminescence, and the mean and standard deviations for the stimulus intensities are shown in Table II. The mean threshold of computed peak water velocity for a jump response was 64 μm s^{-1}, whereas that for eliciting bioluminescence was 5520 μm s^{-1}. The variability of the threshold for bioluminescence was greater than that for the jump. On average the stimulus magnitude had to be 90 times greater to elicit bioluminescent discharge than to produce a weak jump, but this ratio ranged from 30 to 180 in the five experiments. Once we established a threshold for bioluminescence for an experimental animal, we usually were able to elicit bioluminescence multiple times at that stimulus level, sometimes within half an hour from the previous discharge.

Water velocity was not the only stimulus characteristic that affected the likelihood of a bioluminescent discharge, as shown in Table II. Stimulus length was important: the multi-cycle sinusoidal stimulus (F700) was more effective than the 1.5-cycle one (S700; Table II). Furthermore, repeated presentation of stimuli in quick succession was even more effective. In these cases, the animals would respond with only a jump to the first and second stimulus presentations, but would bioluminesce as well as jump to the subsequent one or two stimuli.

Characteristics of evoked bioluminescence

In our tethered animals, bioluminescence typically (though not always) outlasted the jump. The PMT record in Figure 3C shows that bioluminescence was initiated at about 30 ms post-stimulus, corresponding to the second kick. It lasted throughout the recording period, although by 200 ms post-stimulus it was well along an exponential decay. Excerpts from the corresponding video record are shown in Figure 5. Taken at 30 frames per second (fps), with the frame following stimulus delivery tagged by a light flash, the first frame shows no bioluminescence and the onset of the major kick transients occur in this interval. Bioluminescence begins to appear from the region of the abdomen in the next frame, and reaches a peak in the third. Its near-absence from the last two frames is partly a result of decay and partly that much of the material has left the field of view. Thirty-five minutes later a second trial for the same animal as in Figures 3 and 5 elicited an escape as well as a bioluminescent discharge from both head and abdomen (Fig. 6). The animal bioluminesced in response to the sec-
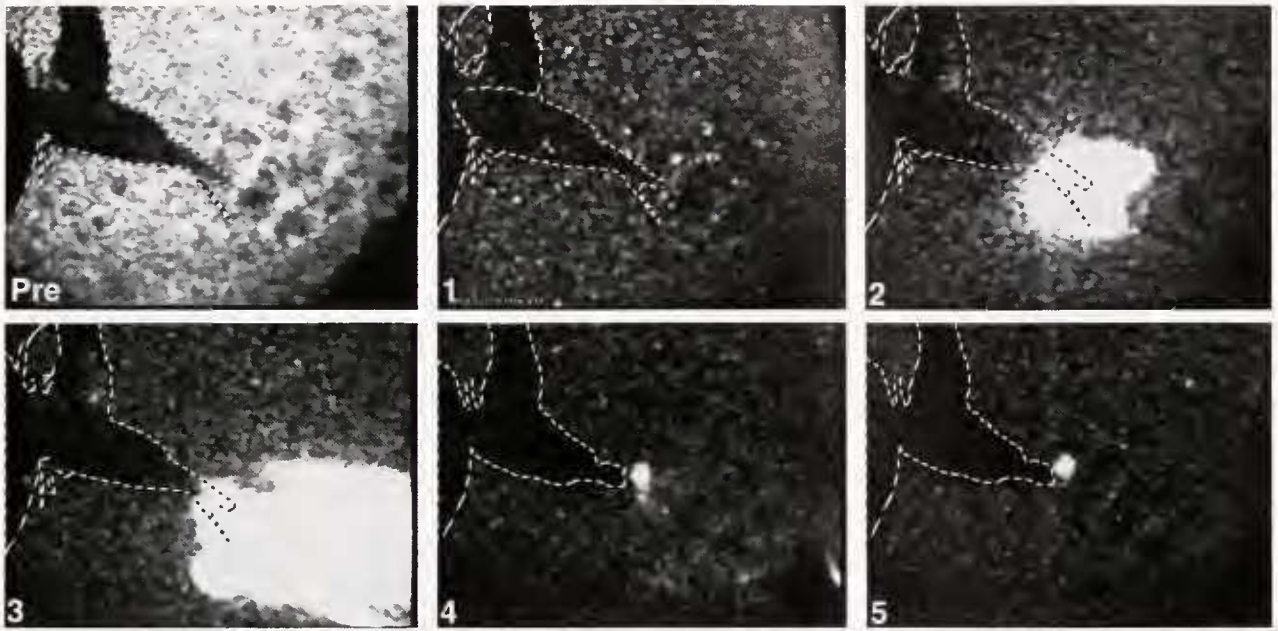
Figure 5. Video frames showing the bioluminescent discharge associated with the records in Figure 3C. The pre-stimulus frame is a composite of the 10 frames preceding stimulus presentation. The next five video stills are from frames 3–7, counting the first post-stimulus frame as 1 (30 fps). Ventral-posterior aspect of animal faces camera. Discharge is primarily from abdominal glands. Broken outlines up to frame 3 indicate position of body prior to stimulation.

ond stimulus of a train of five. It was somewhat more delayed (50-ms latency) and shorter (100-ms duration) than the earlier response (peak amplitude could not be measured owing to saturation of the PMT), but the jump was twice the length (10 kicks *versus* 5).

Records of jumps and bioluminescent discharges from a male *Pleuromamma xiphias* are shown in Figure 7. In this case the animal completed its jump before the bioluminescence. This example was chosen to illustrate a double reaction. The animal responded with two sets of kicks and matching bioluminescent discharges. The discharges were small and short in duration. The animals routinely push the bolus of bioluminescence away from them by flicking their urosomes. This can be seen in Figure 7 as the streaks of bioluminescence move across the screen. The force generated by this behavior is very small compared to the pereiopod power strokes and does not register on the force record.

Comparing this record with the data from the female of Figures 3, 5, and 6 shows the differences that occur when the pereiopods beat during emission of bioluminescent material. The combined kicking and bioluminescence produce the explosion of bioluminescence seen in the video frames. This is in contrast to the male (Fig. 7), in which the luminescent material clung to the urosome, presenting a streaky appearance.

Temporal relations between jump and bioluminescence

The rapid swim was always initiated before the bioluminescence, as illustrated in Figure 8, a scatter plot of jump latencies *versus* bioluminescence latencies. All points are above the line with a slope of one. Rapid swims were initiated within 7 to 20 ms (mean $\pm$ SD = 14 $\pm$ 4 ms), whereas bioluminescence latencies ranged from 20 to 50 ms (with one very delayed response that started at 110 ms; mean $\pm$ SD = 49 $\pm$ 26 ms). In general, the longer the rapid swim latency the greater the delay for the bioluminescence, although the correlation coefficient was not significant (r = 0.508, n = 8). Bioluminescent discharges in response to the hydrodynamic stimulus were typically short, lasting from 50 to 350 ms. Luminescence often (*e.g.,* Figs. 3C; 7), but not always (Fig. 6), extended well after the termination of the jump.

Discussion

Escape jumps

Like all pelagic calanoids, mesopelagic *Pleuromamma xiphias* has an impressive escape jump at its disposal. When sensitivity to water perturbations and jump kinematics measured in tethered animals are compared to similar data for neritic *Undinula vulgaris* (Lenz and Hartline, 1999), a pat-
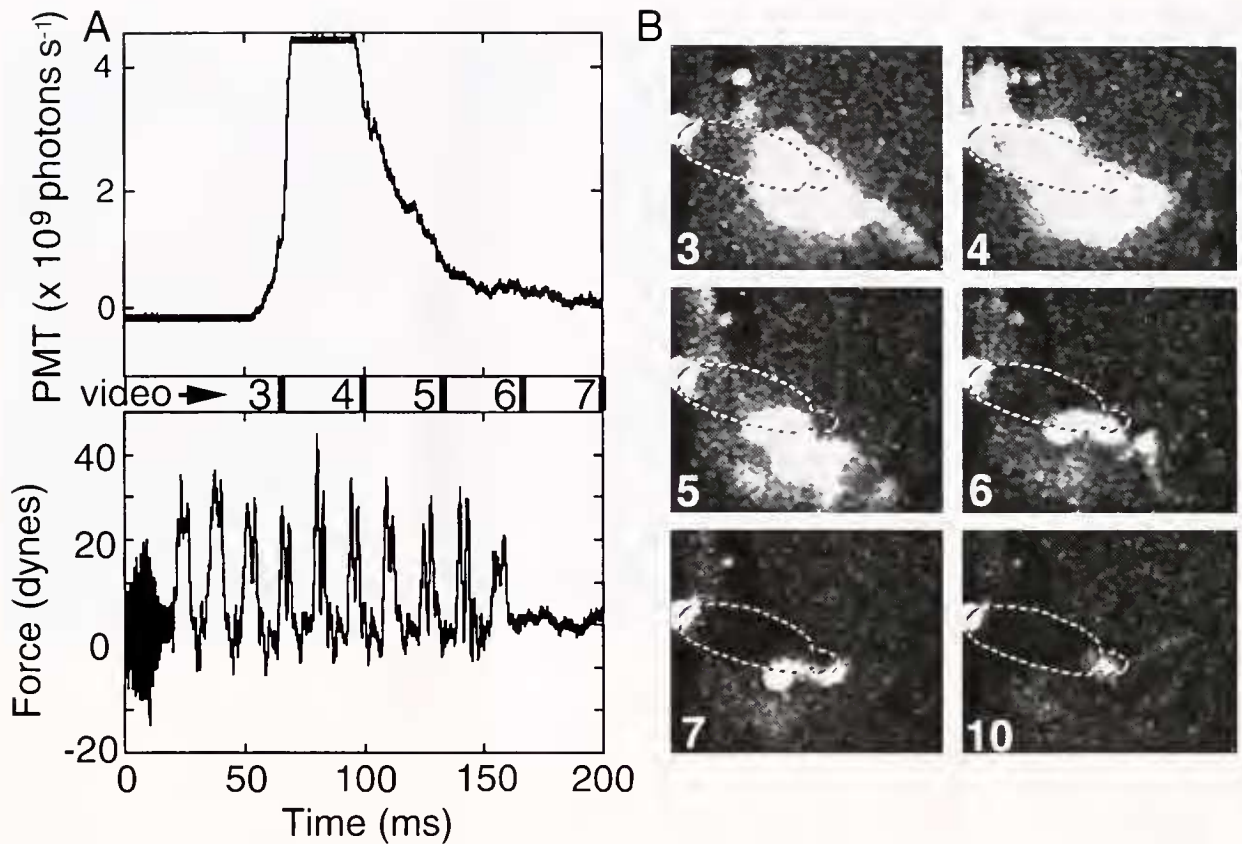
Figure 6. (A) Force record of a second response from the animal in Figures 3 and 5. Comparison shows variability in propulsion and bioluminescence. Note the greater duration of the jump and the shorter duration of the bioluminescent discharge. Stimulus: second in a train of five identical to that for Figures 3 and 5, delivered 35 min following. (B) Video frames 3–7 and 10 following stimulus, showing bioluminescent discharge associated with records of A (30 fps). Broken outline indicates position of body prior to stimulation. Note luminescent discharge from cephalic gland.

tern of characteristics emerges that is similar in broad scope but distinctive in detail. *P. xiphias* sensitivities (~60 μm s^{-1}) are similar to, though perhaps somewhat lower than, those in *U. vulgaris* (~40 μm s^{-1}). Minimum latencies for *P. xiphias* (~6 ms) were distinctly longer than for *U. vulgaris* (~2 ms). This difference in reaction times is in part explained by the lack of myelination of nerve fibers in the Augaptiloidea (Davis *et al.*, 1999). Peak forces of kicks from *U. vulgaris* showed a small gradation in magnitude as a function of the strength of the triggering stimulus and over the course of an escape jump. In contrast, those of *P. xiphias* exhibited a much wider range, with a 5- to 10-fold difference between the weak kicks produced to near-threshold stimuli and the strongest kicks in the middle of a spindle-shaped jump. The strongest kicks registered in our apparatus by *U. vulgaris* (100 dynes) were almost twice the peak forces measured from *P. xiphias* (Table 1). In *U. vulgaris*, the initial one or two kicks were the strongest, whereas in *P.*

xiphias, the strength of kick built up over several cycles, and then waned, giving rise to the spindle-shaped envelope. Although both species produced multiple kicks in response to threshold and well supra-threshold stimuli, *U. vulgaris* consistently produced fewer (2–3 typical; up to 9) than did *P. xiphias* (5–10 typical; up to 35). For comparably sized animals, this should result in longer jump distances in the latter species. This expectation is in agreement with casual observations made while attempting to catch *P. xiphias* in an open vessel: jumps of tens of centimeters are not atypical, while those of *U. vulgaris* are shorter (3 to 5 cm).

Bioluminescent discharges can be evoked by hydrodynamic stimuli

Pleuromamma xiphias will produce a bioluminescent discharge to a brief water disturbance; tactile stimulation is not
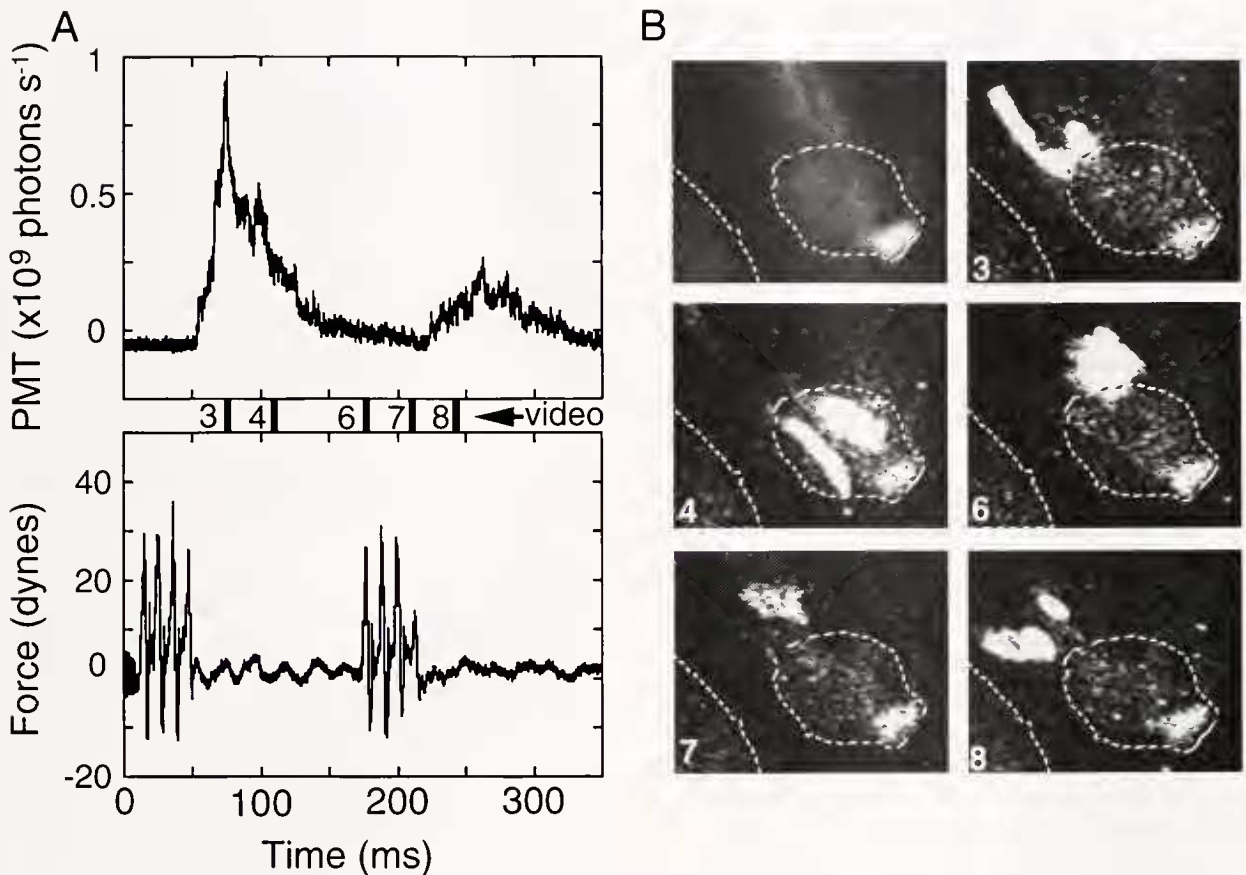
Figure 7. Response of *Pleuromamma xiphias* adult male to a large hydrodynamic stimulus. (A) PMT and force records. (B) Video frames of bioluminescent discharge. The pre-stimulus frame is a composite of 10 frames preceding stimulus presentation. The next five video stills are post-stimulus frames 3–8. Approximate times of frames are indicated with marks along time axis of A. Note the occurrence of two jumps and two separate bioluminescent discharges, spaced 150 ms apart. Stimulus: 700 Hz, 1.5 cycles, maximum water velocity of 6600 μm s^{-1} at rostrum. Piezoelectric transducer: PZL-060; sphere diameter: 5.1 mm; distance from center of ball to rostrum: 4.8 mm, vertical peak-to-peak movement of sphere: 40 μm. Broken outlines indicate position of body prior to stimulation and a portion of the stimulating sphere in lower left corner; posterior view of animal with dorsal toward lower right corner of frames. Glowing material appears associated with abdominal glands (BPL97-6.D03).

required. The magnitude of the stimulus required varied among experimental animals, but in general was significantly greater than that sufficient to trigger strong escape jumps (velocities of 2000 to 9000 μm s^{-1}). When presented with a threat, *P. xiphias* preferentially responds with an escape jump. However, if the threat is prolonged or persists as in the case of repetitive strong stimulation, then the jump is more likely to be accompanied by a bioluminescent discharge. Widder (1992) found a similar pattern for *Gaussia princeps*. During a train of electrical stimulation (3 s^{-1}) *G. princeps* would respond with an escape alone until the fifth stimulus, when it finally produced a bioluminescent discharge as well.

Bioluminescence is delayed compared to the jump

Bioluminescence was always initiated after the onset of a jump sequence. Although the numbers of animals tested were insufficient for complete reliability, in two animals of our study (both males), jumps were completed before the bioluminescence began. In four others (all females), the bioluminescent discharge commenced during the train of kicks. This resulted in a qualitative difference in the visual effect of the bioluminescence, the luminescent bolus being swept along by water propelled posteriorly by the power strokes. An animal that bioluminesces after it has stopped swimming would seem more likely to become a victim of a predatory attack if the luminescent bolus attracts a predator.
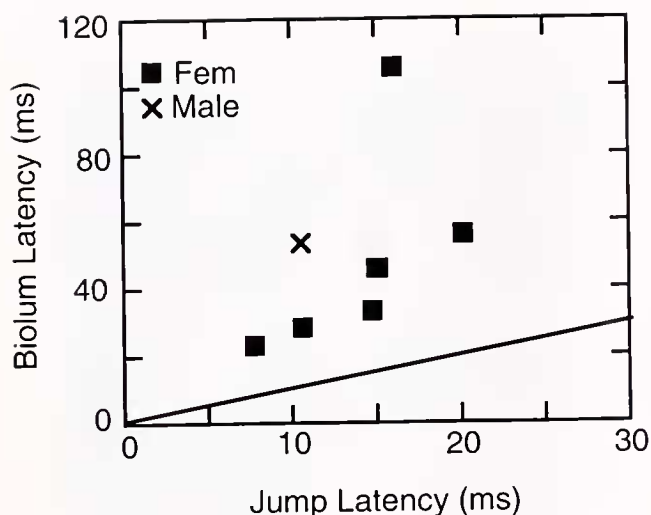
Figure 8. Scatter plot of fast swim latencies (*x* axis) *versus* biolumi-nescence latencies (*y* axis). Squares: adult females (*n* = 7); crosses: adult male (*n* = 1). All points lie above the line, which has a slope of one.

The possibility that there might be a sexual difference in the response patterns is intriguing.

Characteristics of bioluminescence and its relation to other cases reported in the literature

The kinetics and spatial patterns of bioluminescence re-leased by copepods have been studied for copepods stimu-lated with electrical pulses (Latz *et al.*, 1987; Bowlby and Case, 1991) and for copepods stimulated by mechanical disturbance of undefined frequency and intensity (Latz *et al.*, 1990). For the large mesopelagic copepod *Gaussia princeps*, Bowlby and Case (1991) identified three types of flash in response to single electrical stimuli: a fast flash of about 2-s duration, a long flash of 7-s duration, and a slow flash of 17-s duration. Latz *et al.* (1987) found two compo-nents to flashes in *P. xiphias* stimulated with a single electrical pulse: a fast component that reached maximum intensity in < 100 ms and a slow component that reached peak intensity in > 600 ms. Double flashes with fast and slow characteristics were also observed. Using an intensi-fied video system, he observed that the fast component originated from thoracic and abdominal glands, without obvious discharge of bioluminescent material away from the body; the slow component of flashes was caused by the discharge of luminescent fluid from the abdominal organ. Flashes with similar kinetics were observed for *P. xiphias* exposed to mechanical stimulation from a stirring paddle with three tines rotated at 2000 rpm for < 1 s. Since the spatial relationship between the copepod and the rotating tines is unknown during the stimulation period, neither the frequency nor the intensity of mechanical stimulation is

known. In addition to strong hydrodynamic stimulation caused by the velocity of the water and the shear created by the spinning tines, mechanical stimulation is possible through direct contact of the copepods with the tines or by contact with the walls of the scintillation vial following an escape jump. In our observations of bioluminescence evoked by hydrodynamic stimuli of known intensity, only fast flashes were observed. In contrast to the observations of Latz *et al.* (1990), we observed bioluminescence having fast flash kinetics originating from abdominal glands, and with obvious discharge of bioluminescent material away from the body. We have noted that a copepod's ability to produce a second bioluminescent discharge shortly after a previous one is not necessarily precluded. Thus recovery times mea-sured in TMSL protocols (8–24 h) are probably overly long for most natural situations.

Ecological significance

Vertically migrating copepods such as *Pleuromamma xiphias* are important components of mesopelagic food webs, and *Pleuromamma* spp. are often preferred prey of mesopelagic fish (Hopkins and Baird, 1985; Hopkins *et al.*, 1996). To help them avoid predation, these copepods have evolved several defensive behaviors, including vertical mi-gration (Bennett and Hopkins, 1989), strong escape jumps (Buskey *et al.*, 1987; present study), and bioluminescence (Clarke *et al.*, 1962). In contrast to the diversity of strategies possessed by *P. xiphias*, neritic *Undinula vulgaris* appears to have relied on enhancing the speed and strength of the escape response itself as a survival mechanism (Davis *et al.*, 1999; Lenz and Hartline, 1999). The production of light in an otherwise dark environment may at first seem counter-intuitive as a defense mechanism against visual predators; discharge of bioluminescence while the predator is still remote might help the predator locate its prey. However, the higher stimulus threshold for eliciting bioluminescence compared to escape jumps suggests that copepods save this defense for what are perceived to be the strongest threats by predators in close proximity. Mesopelagic predators have sensitive eyes adapted to low light levels, and the discharge of bioluminescence when the predator is nearby may serve to temporarily blind and confuse the predator (Buck, 1978; Morin, 1983). Since copepods initiate escape jumps prior to release of bioluminescence, and leave behind distinct drop-lets or clouds of bioluminescent material (Widder, 1992), the bioluminescent discharge may also serve as a decoy to confuse visual predators (Morin, 1983).

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